09/988,292

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Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L40	L39 and chimeric	15	L40
L39	L36 and (Fab near5 single chain)	19	L39
L38	L36 and (Fab near5 single chain near5 chimeric)	0	L38
L37	L36 and (Fab near5 sigle chain near5 chimeric)	0	L37
L36	antibod$3 near5 composition$1 near5 carrier$1	589	L36
L35	antibod$3 near5 composition$1	7292	L35
L34	antibod$3 near5 compositon near5 carrier	0	L34
L33	L32 and carrier$1	13	L33
L32	L31 and composition$1	15	L32
L31	antibod$3 near5 chimeric near5 Fab near5 single chain	23	L31
L30	l29 and chimeric	1	L30
L29	L7 and hybridoma	8	L29

L28	L7 and Fab	0	L28
L27	L26 and antibod$3	19	L27
L26	lactose binding lectin	19	L26
L25	L24	0	L25
L24	lactose binding lectin (P) antibod$3	0	L24
L23	L22 and single chain	0	L23
L22	L21 and composition41	0	L22
L21	L20 and hybridoma	2	L21
L20	L19 and glycosylat$2	2	L20
L19	L18 and chimer$2	2	L19
L18	L17 and polyclon$	2	L18
L17	L16 and Fab	2	L17
L16	L15 and label$2	4	L16
L15	L14 and monoclon$	8	L15
L14	L13 and human$	8	L14
L13	antibod$3 near5 chiu	8	L13
L12	L10 and monoclon$	0	L12
L11	l10 and glycosylat$2 and monoclon$	0	L11
L10	4468457.pn.	3	L10
L9	l7 and Fab	0	L9
L8	L7 and single chain	0	L8
L7	L6 and label$2	9	L7
L6	L5 and human$	10	L6
L5	L4 and polyclon$	10	L5
L4	L3 and monoclon$	11	L4
L3	l2 and glycosylat$2	13	L3
L2	lactose binding lectins and antibod$3	19	L2

DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ

L1	lactose binding lectins near5 antibod$3	0	L1

END OF SEARCH HISTORY

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L28	L7 and Fab	0	L28
L27	L26 and antibod$3	19	L27
L26	lactose binding lectin	19	L26
L25	L24	0	L25
L24	lactose binding lectin (P) antibod$3	0	L24
L23	L22 and single chain	0	L23
L22	L21 and composition41	0	L22
L21	L20 and hybridoma	2	L21
L20	L19 and glycosylat$2	2	L20
L19	L18 and chimer$2	2	L19
L18	L17 and polyclon$	2	L18
L17	L16 and Fab	2	L17
L16	L15 and label$2	4	L16
L15	L14 and monoclon$	8	L15
L14	L13 and human$	8	L14
L13	antibod$3 near5 chiu	8	L13
L12	L10 and monoclon$	0	L12
L11	l10 and glycosylat$2 and monoclon$	0	L11
L10	4468457.pn.	3	L10
L9	l7 and Fab	0	L9
L8	L7 and single chain	0	L8
L7	L6 and label$2	9	L7
L6	L5 and human$	10	L6
L5	L4 and polyclon$	10	L5
L4	L3 and monoclon$	11	L4
L3	l2 and glycosylat$2	13	L3
L2	lactose binding lectins and antibod$3	19	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	lactose binding lectins near5 antibod$3	0	L1

END OF SEARCH HISTORY

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L29: Entry 5 of 8 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766856 A
TITLE: Diagnostic method for evaluating advanced glycosylation endproducts using MAC-2 receptor

Abstract Paragraph Left (1):
Soluble and membrane associated forms of Mac-2 (also termed Carbohydrate Binding Protein [CBP]-35 and L-34) recognizes and binds to Advanced Glycosylation Endproducts (AGEs) with higher affinity than it binds carbohydrates, such as its "natural" ligand, galactose. The level of soluble Mac-2 in plasma or serum provides a prognostic indicator of the susceptibility of an individual to AGE complications. Thus, the present invention includes various therapeutic and diagnostic utilities predicated on the identification and activities of Mac-2 for binding AGEs. Pharmaceutical compositions of the invention comprise an effective amount of Mac-2 admixed with a pharmaceutically acceptable carrier. Diagnostic utilities include assays such as immunoassays for the presence and amount of Mac-2 in a biological sample, e.g., serum or plasma. Such assays can be performed with labeled receptors, antibodies, ligands and other binding partners of Mac-2. The invention further provides screening assays to evaluate new drugs by their ability to promote or inhibit Mac-2 production or activity, as desired. The above assays can be used to detect the presence or activity of invasive stimuli, pathology or injury, the presence or absence of which may affect the structure or function of specific organs. In a specific embodiment, the level of soluble Mac-2 varies between different populations of diabetics, and between diabetics and normals.

Brief Summary Paragraph Right (3):
Since the amount of AGEs found in human tissues is less than could be predicted from protein/glucose incubation studies in vitro, it appeared that there might be normal mechanisms to remove those long-lived proteins which had accumulated AGEs in vivo. Particularly, and as set forth initially in parent application Ser. No. 907,747, now abandoned and the above-referenced applications that have followed, monocytes/macrophages and endothelial cells were found to display high affinity surface binding activity specific for AGE moieties independent of the protein which was AGE-modified. This AGE-receptor was shown to differ from other known scavenger receptors on these cells.

Brief Summary Paragraph Right (6):
The AGE-specific receptor system now includes a variety of tissues and cell types in addition to monocyte/macrophages for which receptor-mediated AGE-protein internalization and digestion was first described. Endothelial and mesangial cells, as well as fibroblasts, have since been shown to specifically bind AGE-modified protein. In macrophages, AGE-protein uptake is accompanied by the release of a variety of potent cytokines and growth factors, which may coordinate processes of normal tissue remodeling. The other cell types do not bind the model compound AGE, FFI, nor are they known to release cytoidnes and growth factors in response to AGE-ligand binding, but each cell type does display distinct functional responses. For example, endothelial cells exhibit enhanced surface procoagulant activity and permeability; and mesangial cells display enhanced matrix protein synthesis; while human fibroblasts increase proliferation upon exposure to AGEs. Thus, it is now well established that the removal of AGE-modified proteins is facilitated through specific cell surface receptors identified first on cells of the monocyte/macrophage lineage (Radoff et al., 1900, Diabetes 39:1510-18) and subsequently on endothelial cells, mesangial cells and fibroblasts (Esposito et al., 1989, J. Exp. Med. 170:1387; Skolnik et al., 1991, J. Exp. Med; Kirstein et al., 1990, J. Cell Biochem. 14E (Suppl.):0224). In addition to the uptake and degradation of AGE-modified proteins by macrophages, studies on the AGE-receptor/ligand interactions have revealed a range of biologically important responses, including chemotaxis, activation, cytokine production, and growth factor

secretion (Skolnik et al., supra; Kirstein et al., supra). The properties have led to the hypothesis that the AGE-R complex plays an important role in normal growth and tissue turnover.

Brief Summary Paragraph Right (13):
Antibodies raised against both p60 and p90 recognized surface determinants on rat monocytes and macrophages. These antibodies inhibited AGE binding and neutralized AGE-dependent responses on human monocytes/macrophages, murine mesangial cells and rat T cells, suggesting that the AGE-receptor system involves highly conserved proteins.

Brief Summary Paragraph Right (14):
The inventors herein are authors or coauthors on many publications relating to AGE receptors, including the following: "FUNCTION OF MACROPHAGE RECEPTOR FOR NONENZYMATICALLY GLYCOSYLATED PROTEINS IS MODULATED BY INSULIN LEVELS", Vlassara, Brownlee and Cerami, DIABETES (1986), Vol. 35 Supp. 1, Page 13a; "RECOGNITION AND UPTAKE OF HUMAN DIABETIC PERIPHERAL NERVE MYELIN BY MACROPHAGES", Vlassara, H., Brownlee, M., and Cerami, A. DIABETES (1985), Vol. 34, No. 6, pp. 553-557; "HIGH-AFFINITY-RECEPTOR-MEDIATED UPTAKE AND DEGRADATION OF GLUCOSE-MODIFIED PROTEINS: A POTENTIAL MECHANISM FOR THE REMOVAL OF SENESCENT MACROMOLECULES", Vlassara H., Brownlee, M., and Cerami, A., PROC. NATL. ACAD. SCI. U.S.A. (Sept. 1985), Vol. 82, pp. 5588-5592; "NOVEL MACROPHAGE RECEPTOR FOR GLUCOSE-MODIFIED PROTEINS IS DISTINCT FROM PREVIOUSLY DESCRIBED SCAVENGER RECEPTORS", Vlassara, H., Brownlee, M., and Cerami, A. JOUR. EXP. MED. (1986), Vol. 164, pp. 1301-1309; "CHARACTERIZATION OF A SOLUBILIZED CELL SURFACE BINDING PROTEIN ON MACROPHAGES SPECIFIC FOR PROTEINS MODIFIED NONENZYMATICALLY BY ADVANCED GLYCOSYLATION END PRODUCTS", Radoff, S., Vlassara, H. and Cerami, A., ARCH. BIOCHEM. BIOPHYS (1988), Vol. 263, No. 2, pp. 418-423; "ISOLATION OF A SURFACE BINDING PROTEIN SPECIFIC FOR ADVANCED GLYCOSYLATION ENDPRODUCTS FROM THE MURINE MACROPHAGE-DERIVED CELL LINE RAW 264.7", Radoff, S., Vlassara, H., and Cerami, A., DIABETES, (1990), Vol. 39, pp. 1510-1518; "TWO NOVEL RAT LIVER MEMBRANE PROTEINS THAT BIND ADVANCED GLYCOSYLATION ENDPRODUCTS: RELATIONSHIP TO MACROPHAGE RECEPTOR FOR GLUCOSE-MODIFIED PROTEINS", Yang, Z., Makita, Z., Horii, Y., Brunelle, S., Cerami, A., Sehajpal, P., Suthanthiran, M. and Vlassara, H., J. EXP. MED., (1991), Vol. 174, pp. 515-24). All of the foregoing publications and all other references cited herein are incorporated by reference.

Brief Summary Paragraph Right (16):
Murine Mac-2 was initially shown to be expressed on the surface of inflammatory macrophages (Ho and Springer, 1982, J. Immunol. 128:1221-28). cDNAs encoding Mac-2 have been obtained. The protein was shown to be secreted and to have the characteristics of a galactose-specific lectin (Cherayil et al., 1989, J. Exp. Med. 170:1959-72); a human Mac-2 lectin specific for galactose has also been cloned (Cherayil et al., 1990, Proc. Natl. Acad. Sci. USA 87:7324-28). The murine protein was independently identified on the basis of carbohydrate binding as carbohydrate-binding protein 35 (CBP 35) in mouse fibroblasts, and on the basis of nuclear localization (Jia and Wang, 1988, J. Biol. Chem. 263:6009-11; Moutsatsos et al., 1987, Proc. Natl. Acad. Sci. USA 84:6452-56). The same protein (named L-34) has also been identified as a tumor cell surface lectin (Raz et al., 1989, Cancer Res. 49:3489-93). Both Mac-2 and a highly similar rat cytosolic protein have the ability to bind murine IgE (Liu et al, 1985, Proc. Natl. Acad. Sci. USA 82:4100-04; Cherayil et al., 1989, supra). Mac-2 has also been found to bind to laminin (Woo et al., 1990, J. Biol. Chem. 265:7097-99, Cherayil et al., 1990, supra). Mac-2, and other S-type lectins, are found in the nucleus, where these proteins are responsible for binding of various neoglycoproteins (Wang et al., 1991, Glycobiology 1:243-252). Under the auspices of an international agreement, Mac-2 is now referred to as galactin-3 (Barondes et al., 1994, Cell 76:597-8).

Brief Summary Paragraph Right (23):
Diagnostic utilities include assays such as immunoassays for the presence and amount of Mac-2 in a biological sample, e.g., serum or plasma. Such assays can be performed with labeled receptors, antibodies, ligands and other binding partners of Mac-2. The invention further provides screening assays to evaluate new drugs by their ability to promote or inhibit Mac-2 production or activity, as desired. The above assays can be used to detect the presence or activity of invasive stimuli, pathology or injury, the presence or absence of which may affect the structure or function of specific organs.

Brief Summary Paragraph Right (24):
The ligands useful in the diagnostic procedures of the present invention are generally AGE derivatives that bind to AGE binding partners. Suitable ligands are selected from the reaction products of reducing sugars, such as glucose and glucose-6-phosphate (G6P), fructose and ribose. These sugars are reactive with peptides, proteins and other biochemicals such as BSA, avidin, biotin, and enzymes such as alkaline phosphatase. The ligands can be labeled, or attached to a solid phase support, for use in assays to detect the presence of, and, if desired, measure the amount of, Mac-2 in a sample suspected of containing Mac-2.

Drawing Description Paragraph Right (2):
FIG. 1 depicts an immunoblot analysis that shows that anti-P90 recognizes recombinant Mac-2. Purified, recombinant Mac-2 (rMac-2) was subjected to SDS-PAGE and Western blot analysis. Normal rat sera (Lane A, NRS) and polyclonal antisera specific for a synthetic N-terminal peptide of the p90 AGE receptor (Lane C, Anti-Np90) did not react with rMac-2; anti-Mac-2 monoclonal antibody M3/38 (Ho and Springer, 1982, J. Immunol. 128:1221-28) (Lane B, Anti Mac-2), and polyclonal antisera raised against intact p90 (Yang et al., 1991, J. Exp. Med. 174:515-24) (Lane D, Anti p90) reacted with Mac-2.

Drawing Description Paragraph Right (3):
FIG. 2 depicts a graph showing the saturability of AGE binding to Mac-2. Binding of increasing concentrations of .sup.125 I-labeled BSA-AGE to 3 .mu.g of purified rMac-2 immobilized on a nitrocellulose membrane (.quadrature.) was measured. After washing, the bound radioactivity was measured by phosphoimage analysis. Binding of the highest concentration of labeled AGE-BSA was completely abrogated by competition with a 50-fold excess of cold AGE-BSA (.diamond.).

Drawing Description Paragraph Right (4):
FIG. 3 depicts a blot analysis of anti-Mac-2 immunoprecipitates of several AGE-binding proteins. Panel A. Detergent cell extracts were prepared from RAW 264.7 cells. After preclearing with BSA-SEPHAROSE, immunoprecipitations were performed using isotype control (Lane A), or anti-Mac-2 monoclonal antibody M3/38 (Lane B). After SDS-PAGE and transfer to nitrocellulose, the blots were probed with .sup.125 I-labeled AGE-BSA. Panel B. A purified carboxyl-terminal fragment of Mac-2 was subjected to ligand blot analysis in the absence (Lane A) or presence (Lane B) of a 50-fold excess of cold AGE-BSA.

Drawing Description Paragraph Right (5):
FIG. 4 depicts the ability of various putative ligands to compete with .sup.125 I-labeled AGE-BSA for binding to Mac-2 from whole cell extracts. After immunoprecipitation with anti-Mac-2 MAb, the precipitate was subjected to ligand blot analysis with 300 nM .sup.1251 -AGE-BSA and either no inhibitor (a), or in the presence of a 100 mM (300,000-fold excess) of lactose (b), galactose (c), galactosamine (d), glucosamine (e), Amadori-BSA (f; the concentration of Amadori-BSA was 1.5 mM, a 500-fold excess), cold AGE-BSA (g; the concentration of cold AGE-BSA was 1.5 mM, or a 50-fold excess), or FFI-human albumin (HA) (h; the concentration of FFI-HA was 1.5 mM) competitors. The level of bound radioactivity was measured using phosphoimage analysis.

Drawing Description Paragraph Right (6):
FIG. 5 presents an autoradiogram of immunoprecipitated or ligand-affinity precipitated, cell surface iodinated RAW 264.7 macrophage cells, after SDS-PAGE. Cell surface proteins were radiolabeled using the lactoperoxidasecatalyzed iodination technique. Detergent extracts were prepared and were subjected to immunoprecipitation with rat IgG.sub.2a isotype control (Lane A), anti-Mac-2 monoclonal antibody M3/38 (Lane B), or AGE-BSA conjugated to SEPHAROSE (ligand affinity precipitation) (Lane C). The precipitated proteins were subjected to SDS-PAGE and were visualized by autoradiography.

Detailed Description Paragraph Right (8):
The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness, and the like, when administered to a human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the

U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "pharmaceutically acceptable carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solutions, such as saline solutions and aqueous dextrose and glycerol solutions, are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

Detailed Description Paragraph Right (34):
The present invention also relates to a variety of diagnostic applications, including methods for the measurement of the presence and amount of Mac-2 in animals, including humans. The methods comprise assays involving in addition to the analyte, one or more binding partners of Mac-2.

Detailed Description Paragraph Right (38):
The present invention also relates to a method for detecting the presence of stimulated, spontaneous, or idiopathic pathological states in mammals, by measuring the corresponding presence of Mac-2. In one aspect of the invention, the stimulated, spontaneous, or idiopathic pathological state is induced by phagocytic cell, e.g., macrophage, activation against AGEs. More particularly, the activity of macrophages against AGEs may be followed directly by assay techniques such as those discussed herein, through the use of an appropriately labeled quantity of at least one of the binding partners to Mac-2 as set forth herein.

Detailed Description Paragraph Right (39):
Thus, both Mac-2 and any binding partners thereto that may be prepared, are capable of use in connection with various diagnostic techniques, including immunoassays, such as a radioimmunoassay, using for example, a receptor or other ligand to an AGE that may either be unlabeled or if labeled, then by either radioactive addition, reduction with sodium borohydride, or radioiodination.

Detailed Description Paragraph Right (40):
In an immunoassay, a control quantity of a binding partner to Mac-2 may be prepared and optionally labeled, such as with an enzyme, a compound that fluoresces and/or a radioactive element, and may then be introduced into a tissue or fluid sample of a mammal believed to be expressing Mac-2. After the labeled material has had an opportunity to react with sites within the tissue, the resulting mass may be examined by known techniques, which may vary with the nature of the label attached. Preferably if such in vivo detection is desired, the radio-label is technetium-99 (.sup.99 Tc), or a magnetic shift reagent label such as gadolinium or manganese, is used as a label.

Detailed Description Paragraph Right (41):
The presence of Mac-2 activity in animals can be ascertained in general by immunological procedures, which utilize either a binding partner to the Mac-2, or a ligand thereto, or combinations thereof, in which one component is labeled with a detectable label. In a preferred aspect, the presence of Mac-2 is ascertained in an assay that involves an antibody Ab, labeled with a detectable label, or an antibody Ab.sub.2 labeled with a detectable label, or a chemical conjugate with a binding partner to Mac-2 labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the molecular species is labeled, and "M" in this instance stands for Mac-2 under examination:

Detailed Description Paragraph Right (42):
These general procedures and their application are all familiar to those skilled in the art and are presented herein as illustrative and not restrictive of procedures that may be utilized within the scope of the present invention. The "competitive" procedure, Procedure A, is described in U.S. Pat. Nos. 3,654,090 and 3,850,752. Optional procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043, while optional procedure D is known as the "double antibody", or "DASP" procedure.

Detailed Description Paragraph Right (43):

A further alternate diagnostic procedure employs multiple labeled compounds in a single solution for simultaneous radioimmunoassay. In this procedure disclosed in U.S. Pat. No. 4,762,028 to Olson, a composition may be prepared with two or more analytes in a coordinated compound having the formula: radioisotope-chelator-analyte.

Detailed Description Paragraph Right (44):
In each instance, Mac-2 forms complexes with one or more binding partners and one member of the complex may be labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by the known applicable detection methods.

Detailed Description Paragraph Right (45):
With reference to the use of an anti-Mac-2 antibody as a binding partner, it will be seen from the above that a characteristic property of Ab.sub.2 is that it will react with Ab.sub.1. This is because Ab.sub.1 raised in one mammalian species has been used in another species as an antigen to raise the antibody Ab.sub.2. For example, Ab.sub.2 may be raised in goats using rabbit antibodies as antigens. Ab.sub.2 therefore would be anti-rabbit antibody raised in goats. Where used and for purposes of this description, Ab.sub.1 will be referred to as a primary or anti-Mac-2 antibody, and Ab.sub.2 will be referred to as a secondary or anti-Ab.sub.1 antibody.

Detailed Description Paragraph Right (46):
The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

Detailed Description Paragraph Right (47):
Suitable radioactive elements may be selected from the group consisting of .sup.3 H, .sup.14 C, .sup.32 P, 35S, .sup.36 Cl, .sup.51 Cr, .sup.57 Co, .sup.58 Co, .sup.59 Fe, .sup.90 Y, 125I, .sup.131 I, and .sup.186 Re. In the instance where a radioactive label, such as one of the isotopes listed above, is used, known currently available counting procedures may be utilized to detect or quantitate the amount of label.

Detailed Description Paragraph Right (48):
In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, thermometric, amperometric or gasometric techniques known in the art. The enzyme may be conjugated to the advanced glycosylation endproducts, their binding partners or carrier molecules by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Also, and in a particular embodiment of the present invention, the enzymes themselves may be modified into advanced glycosylation endproducts by reaction with sugars as set forth herein.

Detailed Description Paragraph Right (50):
A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

Detailed Description Paragraph Right (51):
In specific embodiments, infra, a monoclonal antibody and a polyclonal antibody to Mac-2 are labeled and used to detect the presence, and quantitate the amount, of Mac-2 in a sample. In another specific embodiment, a ligand--AGE-BSA--to Mac-2 is labeled and used to detect the presence, and quantitate the amount, of Mac-2 in a sample. In these specific embodiments, the detectable label is .sup.125 I.

Detailed Description Paragraph Right (52):
Various assay formats are also contemplated by the present invention for detecting the presence, and if desired, the amount, of Mac-2. For example, a direct "sandwich"-type ELISA can be performed, in which an AGE is attached to the solid phase support, and labeled anti-Mac-2 antibody is used to detect binding of Mac-2 to the solid phase AGE. Alternatively, a Mac-2 antibody can be attached to the solid phase support, and a labeled second anti-Mac-2 antibody that does not compete with the first for binding to Mac-2, or a labeled AGE, can be used to detect binding of Mac-2 to the solid phase.

Detailed Description Paragraph Right (53):

Blotting formats, in which all the proteins from a sample are blotted, e.g., by electroblotting, on a solid support, such as nitrocellulose, for detecting the presence, and if desired, the amount of Mac-2 are also contemplated by the instant invention. In a specific embodiment, infra, after blotting the proteins in a sample on nitrocellulose, Mac-2 is detected using a labeled AGE and a labeled antibody to Mac-2.

Detailed Description Paragraph Right (55):
The present invention includes assay systems that may be prepared in the form of test kits for the quantitative analysis of the extent of the presence of advanced glycosylation endproducts. The system or test kit may comprise a labeled component prepared by one of the radioactive and/or enzymatic techniques discussed herein, coupling a label to a binding partner to Mac-2, such as an antibody or ligand, as listed herein; and one or more additional immunochemical reagents, at least one of which is capable either of binding with the labeled component, its binding partner, one of the components to be determined or their binding partner(s).

Detailed Description Paragraph Right (56):
In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of Mac-2. Such kits can also be used to determine the amount of Mac-2 in a sample. In accordance with the testing techniques discussed above, one class of such kits will contain at least labeled antibody to or ligand for Mac-2, and may include directions, depending upon the method selected, e.g., "competitive", "sandwich", "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Detailed Description Paragraph Right (57):
For example, a first assay format contemplates a bound ligand to which is added the analyte, followed by a labeled antibody to Mac-2. The resulting substrate is then washed, after which detection proceeds by the measurement of the amount of labeled antibody specifically retained on the ligand-modified substance.

Detailed Description Paragraph Right (59):
(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of an anti-Mac-2 antibody or a Mac-2 ligand to a detectable label;

Detailed Description Paragraph Right (63):
(a) a known amount of a binding partner to Mac-2 as described above, such as an antibody or ligand, generally bound to a solid phase to form an immunosorbent, or in the alternative, bound to a suitable tag, or plural such end products, etc. (or their binding partners) one of each;

Detailed Description Paragraph Right (68):
(a) a labeled component which has been obtained by coupling the binding partner of Mac-2 to a detectable label;

Detailed Description Paragraph Right (72):
Both polyclonal and monoclonal antibodies to Mac-2 are contemplated, the latter capable of preparation by well known techniques such as the hybridoma technique, utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Immortal, antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Specific polyclonal antibodies can be raised. Naturally, these antibodies are merely illustrative of antibody preparations that may be made in accordance with the present invention.

Detailed Description Paragraph Right (74):
In one embodiment, Mac-2 is used to coat a solid phase support. The solid phase is then contacted with a sample and a labeled AGE. The presence of AGEs in the sample is detected by their ability to compete with a labeled AGE for binding to solid phase Mac-2. Since carbohydrate binding does not affect Mac-2 binding to AGE (as shown in an Example, infra), this assay is specific for Mac-2.

Detailed Description Paragraph Right (75):

In another embodiment, an AGE can be attached to a solid phase support. The solid support is then contacted with a sample and labeled Mac-2. The presence of AGEs in the sample is detected by their ability to compete with the solid phase AGE for binding to labeled Mac-2. This assay is also specific for detection of AGEs.

Detailed Description Paragraph Right (76):
In a further embodiment, Mac-2 can be used as either a solid phase reagent or the labeled reagent in a direct sandwich ELISA assay for AGEs. The other AGE binding partner should be an anti-AGE antibody, or alternatively another receptor that binds only AGEs.

Detailed Description Paragraph Right (77):
Any of the assay formats, labels, and kits discussed above can be applied to this aspect of the invention as well.

Detailed Description Paragraph Right (80):
In the process of screening an expression library using antibody to one of the components of the AGE-R complex (p90), a partial cDNA clone was isolated with homology to the macrophage surface marker Mac-2, also known as carbohydrate-binding protein (CBP). Mac-2 is a cellular and/or secreted protein of 32 kD. To understand the relationship of Mac-2 to the AGE-R complex, the AGE-binding properties of the molecule were investigated. Purified recombinant rat Mac-2 (rMac-2) bound .sup.125 I-AGE-BSA with saturable kinetics (Kd 3.5.times.10.sup.7 M.sup.-1 and was recognized by antibody to AGE-binding protein p90. Immunoprecipitation of whole cell extracts prepared from RAW 264.7 cells with anti-Mac-2 Mab M3/38, followed by .sup.125 I-AGE-BSA ligand blot revealed several Mac-2 associated proteins (30 kD, 35 kD and 50 kD) with AGE-binding activity. Mac-2 binding of .sup.25 I-AGE-BSA was weakly inhibited by a large excess of several known Mac-2 ligands, e.g., lactose but was fully blocked by cold AGE-BSA.

Detailed Description Paragraph Right (87):
Ligand and Western blotting. Pure recombinant Mac-2 (Agrwal et al. supra) or cell membrane preparations were mixed with an equal volume of Laemli 2 X SDS-PAGE sample buffer containing 5 % .beta.-mercaptoethanol and electrophoresed on a 12% SDS-PAGE. Proteins were then electroblotted onto a nitrocellulose filter, as previously described (Towbin et al., 1979, Proc. Natl. Acad. Sci. USA 76:4350-54). For ligand blot analysis, following blocking for 1 hour in a solution of PBS 5 containing 1.5% BSA and 0.1% Triton -100, 1 mM MgCl.sub.2 and 1 mM CaCl.sub.2 (blocking solution), the nitrocellulose filters were probed with .sup.125 I-labeled AGE-BSA (300 nM) in blocking solution, in the presence or absence of a 50-fold excess of unlabelled AGE-BSA. The blots were washed 3 times with PBS containing 0.1% Triton X-100 and exposed to Kodak XAR-5 film at -80.degree. C. Quantitation of bound radioactivity was performed on a Molecular Dynamics phosphorimager and the values were expressed as relative phosphorimage units.

Detailed Description Paragraph Right (88):
For western blot analysis, following blocking with PBS containing 3% non-fat dry milk, electroblotted proteins were probed with various primary antibodies as indicated in the Results Section, infra, and visualized by using alkaline phosphatase-conjugated secondary antibodies and the NBT-BCIP western blot detection method (Sambrook et al., supra).

Detailed Description Paragraph Right (89):
Antibodies. Rabbit antisera were raised against a synthetic peptide corresponding to the published NH2-terminal amino acid sequence of a rat liver AGE-binding proteins P90 (Yang et al., 1991, J. Exp. Med. 174:515-24). This antisera was designated anti-Np90. Avian anti-rat polyclonal antibodies, raised against purified rat liver AGE-binding proteins p90, were prepared as described (Yang et al., supra). Rat monoclonal antibody (MAb) specific to murine Mac-2 was purified using protein G-sepharose column (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) from culture supernatants of hybridoma M3/38 (ATCC accession number TIB166) as described (Akerstrom et al, 1985, J. Immunol. 135:2589-92). Isotypic control rat lgG2a was purchased from Zymed Immunochemicals (South San Francisco, Calif.).

Detailed Description Paragraph Right (93):
Antibody to the AGE-binding protein p90 recognizes Mac-2. To begin exploring the

relationship between the AGE-receptor (AGE-R) proteins and Mac-2, Western blot analysis was performed using recombinant purified rat Mac-2 (rMac-2) and avian polyclonal antibodies raised either against the purified 90 kD subunit of the rat liver membrane AGE-R (anti-p90) or against the NH.sub.2 -terminal sequence of p90 (anti-Np90). A single immunoreactive species of 32 KD was readily observed using antibodies to the purified intact p90 (anti-p90) or to rMac-2 (M3/38) FIG. 1, lanes B and D), but not by the antibody to the synthetic N-terminal peptide sequence or by the isotypic control rat IgG2a (FIG. 1, lanes C and A). This was consistent with the possibility that p90 contains an epitope shared by Mac-2 or that p90 itself consists of more than one polypeptide, one of which is Mac-2.

Detailed Description Paragraph Right (95):
Similar AGE-binding activity was exhibited by Mac-2 immunoprecipitated from cell membrane extracts from the macrophage-like RAW 264.7 cell line using monoclonal antibody M3/38. Anti-Mac-2-reactive material was subjected to SDS-PAGE and, after transfer to nitrocellulose, to Western and ligand blot analysis. Three proteins with apparent molecular weights of 30 KD, 35 KD and 50 KD were precipitated by M3/38. All three proteins displayed .sup.125 I-AGE-binding activity on ligand blotting (FIG. 3A, lane B). AGE-binding activity was also shown by an 18 kD carboxyl-terminal fragment of Mac-2 (FIG. 3B, lane A), and this binding was completely abrogated in the presence of a 50-fold excess unlabeled AGE-BSA (FIG. 3B, lane B). By comparison, isotypic 1gG2a antibody failed to precipitate any AGE-binding species (FIG. 3A, lane A). The identity of the 35 KD polypeptide as Mac-2 was confirmed by polyclonal anti-CBP35 antibodies (data not shown).

Detailed Description Paragraph Right (97):
Cell-surface Mac-2 binds AGE-BSA. Mac-2 has been found as a cytoplasmic, nuclear and, to a lesser extent, as membrane-associated protein. To demonstrate that cell surface Mac-2 is capable of binding AGE-ligands, surface-iodinated macrophage-like RAW 264 cell extracts were used either for immunoprecipitation using M3/38 mAb, or for ligand-affinity precipitation studies, using an AGE-BSA-sepharose affinity system, as described (Yang et al., 1991, supra). In either case, the protein complexes obtained were eluted with SDS-PAGE sample buffer and electrophoresed. As shown in FIG. 5, lane B, autoradiography of the labelled cell-surface complexes immunoprecipitated by M3/38 monoclonal antibody revealed a polypeptide of approximately 32 kD. This was similar to a polypeptide band obtained by ligand-affinity precipitation of the same extracts, exhibiting identical mobility to Mac-2 (FIG. 5, lane C). In addition, using either procedure (immuno- and ligand-affinity precipitation), a polypeptide with the apparent molecular weight of 90 kD was identified (FIG. 5, upper arrow).

Detailed Description Paragraph Right (98):
This Example provides evidence that the macrophage surface marker Mac-2 has a novel property of binding AGES, and this is unexpectedly a member of the AGE-R protein family. Previously, two polypeptides, designated p60 and p90, were identified as AGE-binding proteins, which may comprise portions of an AGE-R complex. A partial cDNA clone corresponding to Mac-2 was isolated while attempting to clone p90 using a protein expression library and antibody recognition of expressed proteins. Western analysis of rMac-2 was performed using antibodies made either to the purified rat liver membrane p90 polypeptide (anti-p90) or to the N-terminal peptide sequence obtained from the respective analysis of this material (anti-N90). The anti-p90 polyclonal antibody recognized Mac-2, suggesting either an immunologic crossreactivity between p90 and Mac-2 or the presence of anti-Mac-2 immunoreactivity in the original antibody preparations.

Detailed Description Paragraph Right (101):
In agreement with previous reports, the presence of Mac-2 on the cell surface was demonstrated by immunoprecipitation of surface-labeled RAW 264.7 cells. Anti-Mac-2 precipitated the expected 32 kD polypeptide, as well as several other molecules with the apparent molecular weights of 25 kD and 50 kD. In addition, affinity precipitation using AGE-BSA-sepharose yielded two polypeptides, one with identical mobility to Mac-2 at 32 KDa, and a 90 KDa polypeptide.

Detailed Description Paragraph Right (107):
Screening of human diabetic sera (n=22) using the SDS-PAGE-ligand blot analysis described above revealed a 32 kD polypeptide band, migrating identically to Mac-2,

which exhibited an average of 5-fold greater AGE-binding activity on ligand blots compared to normal controls (n=13) (p<0.005). These data are shown in FIG. 6. These data clearly demonstrate that binding of a putative Mac-2 protein with .sup.125 I-AGE-BSA in samples from both Type-I and Type-II diabetics is much greater than in samples from normal controls.

Detailed Description Paragraph Type 1 (1):
(i) a ligand capable of binding with the labeled component (a);

Detailed Description Paragraph Type 1 (2):
(ii) a ligand capable of binding with a binding partner of the labeled component (a);

Other Reference Publication (2):
Sparrow et al, "Multiple Soluble .beta.-Glactoside-binding Lectins from Human Lung" J. Biol. Chem. 262 (15)7383-7390.

Other Reference Publication (4):
Huflejt et al, "L-29, a Soluble Lactose-binding Lectin . . . " J. Biol. Chem. 268 (35):26712-26718.

Other Reference Publication (6):
Wollenberg et al, "Human Keratmocytes Release . . . (IgE-Binding Protein) . . . " J. Exp. Med. 178: 777-785.

CLAIMS:

4. The method according to claim 1, 2, or 3, wherein the mammal is a human.

10. The method according to claim 8 wherein the mammal is a human.

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L40: Entry 11 of 15 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090382 A
TITLE: Human antibodies that bind human TNF.alpha.

Brief Summary Paragraph Right (3):
In an attempt to overcome the problems associated with use of fully-murine antibodies in humans, murine anti-hTNF.alpha. antibodies have been genetically engineered to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains are human-derived, have been prepared (Knight, D. M, et al. (1993) Mol. Immunol. 30:1443-1453; PCT Publication No. WO 92/16553 by Daddona, P. E., et al.). Additionally, humanized antibodies, in which the hypervariable domains of the antibody variable regions are murine-derived but the remainder of the variable regions and the antibody constant regions are human-derived, have also been prepared (PCT Publication No. WO 92/11383 by Adair, J. R., et al.). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction, especially when administered for prolonged periods, e.g., for chronic indications, such as rheumatoid arthritis (see e.g., Elliott, M. J., et al. (1994) Lancet 344:1125-1127; Elliot, M. J., et al. (1994) Lancet 344:1105-1110).

Brief Summary Paragraph Right (4):
A preferred hTNF.alpha. inhibitory agent to murine mAbs or derivatives thereof (e.g., chimeric or humanized antibodies) would be an entirely human anti-hTNF.alpha. antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods. Human monoclonal autoantibodies against hTNF.alpha. have been prepared using human hybridoma techniques (Boyle, P., et al. (1993) Cell. Immunol. 152:556-568; Boyle, P., et al. (1993) Cell. Immunol. 152:569-581; European Patent Application Publication No. 614 984 A2 by Boyle, et al.). However, these hybridoma-derived monoclonal autoantibodies were reported to have an affinity for hTNF.alpha. that was too low to calculate by conventional methods, were unable to bind soluble hTNF.alpha. and were unable to neutralize hTNF.alpha.-induced cytotoxicity (see Boyle, et al.; supra). Moreover, the success of the human hybridoma technique depends upon the natural presence in human peripheral blood of lymphocytes producing autoantibodies specific for hTNF.alpha.. Certain studies have detected serum autoantibodies against hTNF.alpha. in human subjects (Fomsgaard, A., et al. (1989) Scand J. Immunol. 30:219-223; Bendtzen, K., et al. (1990) Prog. Leukocyte Biol. 10B:447-452), whereas others have not (Leusch, H-G., et al. (1991) J. Immunol. Methods 139:145-147).,

Detailed Description Paragraph Right (30):
the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

Detailed Description Paragraph Right (63):
The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as

wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Detailed Description Paragraph Right (84):
Tumor necrosis factor has been implicated in playing a role in the pathophysiology of a variety of autoimmune diseases. For example, TNF.alpha. has been implicated in activating tissue inflammation and causing joint destruction in rheumatoid arthritis (see e.g., Tracey and Cerami, supra; Arend, W. P. and Dayer, J-M. (1995) Arth. Rheum. 38:151-160; Fava, R. A., et al. (1993) Clin. Exp. Immunol. 94:261-266). TNF.alpha. also has been implicated in promoting the death of islet cells and in mediating insulin resistance in diabetes (see e.g., Tracey and Cerami, supra; PCT Publication No. WO 94/08609), TNF.alpha. also has been implicated in mediating cytotoxicity to oligodendrocytes and induction of inflammatory plaques in multiple sclerosis (see e.g., Tracey and Cerami, supra). Chimeric and humanized murine anti-hTNF.alpha. antibodies have undergone clinical testing for treatment of rheumatoid arthritis (see e.g., Elliott, M. J., et al. (1994) Lancet 344:1125-1127; Elliot, M. J., et al. (1994) Lancet 344:1105-1110; Rankin, E. C., et al. (1995) Br. J. Rheumatol. 34:334-342).

Detailed Description Paragraph Right (96):
Tumor necrosis factor has been implicated in the pathophysiology of inflammatory bowel disorders (see e.g., Tracy, K. J., et al. (1986) Science 234:470-474; Sun, X-M., et al. (1988)J. Clin. Invest. 81:1328-1331; MacDonald, T. T., et al. (1990) Clin. Exp. immunol. 81:301-305). Chimeric murine anti-hTNF.alpha. antibodies have undergone clinical testing for treatment of Crohn's disease (van Dullemen, H. M., et al. (1995) Gastroenterology 109:129-135). The human antibodies, and antibody portions, of the invention, also can be used to treat intestinal disorders, such as idiopathic inflammatory bowel disease, which includes two syndromes, Crohn's disease and ulcerative colitis.

Other Reference Publication (6):
Elliot, M.J. et al., "Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor .alpha.", Arthritis & Rheumatism, vol. 36, No. 12, pp. 1681-1690 (1993).